Everglades REMAP Study IV
Detailed Stepwise Sampling Protocol

September 20, 2013

Navigate to the station using the Garmin. The crew member in the co-pilot's seat should communicate with the pilot, who will assess whether it is safe to land at or within a 5 m radius of the coordinates ("nominal"). If not, try to find an alternate location within a 20 m radius, in the same habitat as the original coordinates ("shifted"). Upon identification of a suitable landing site, the pilot should land the helicopter so that the left side pontoon provides direct access to the coordinates (or shifted location).

If there are no suitable locations within that radius, the site is then removed from the list ("rejected"), and you should proceed to the next sampling location. Remember to fill out a data sheet for the rejected station once you land at the next station. Include the Station, Crew, Location and Vegetation Type information, and write in the notes section why the site was rejected.

DUPLICATE STATIONS

If the Station ID ends in a "5," the station is a duplicate. At duplicate stations, all measurements and samples must be collected twice, with the exception of DOC samples (ORANGE VOA bottles). Three DOC samples per replicate are needed, for a total of 6 ORANGE bottles at duplicate stations. Follow instructions for each Roman numeral (Section) on the stepwise sheet, and repeat each section immediately after completing the first time through. Include all steps, even rinsing equipment and donning new, clean gloves. Sections I (Site Photos), VI (Location), and IX (Aerial Photograph) do not need to be repeated. Fill out a 2nd data sheet with duplicate sample information and measurements, and circle Y for duplicate station on both data sheets for that station.

BEFORE ENTERING WATER

Collect all water samples and sonde water chemistry measurements before disturbing the site, working from the pontoons and the helicopter cabin.

****ALL SAMPLERS MUST WEAR GLOVES DURING SAMPLING****
****BE SURE TO RINSE ALL EQUIPMENT WITH AMBIENT SITE WATER AT EACH STATION****
****DON'T TRAMPLE WHERE YOU SAMPLE!****
****SAVE ALL TRASH FROM EACH STATION IN LABELED, SEALED BAGS****

I. PHOTOS

Take a photo of the Station ID sheet (story board) with all information filled in for that site (using label tape and Sharpie). Take a photo of the area to be sampled, off the left pontoon ("ground view"). Check that the photos are in focus and record the photo numbers in the logbook. [On the abbreviated stepwise sheet, "verify/#" is shorthand for checking photographs to make sure they are in focus and composed properly, with minimal glare or backlighting, then recording the photo number(s) in the logbook.]

Standing on the pontoons, take a series of panoramic photos at all cardinal and ordinal directions. Use a compass. Start at NORTH, continue clockwise, and end with another photo at NORTH. This will be a total of 9 photos: N, NE, E, SE, S, SW, W, NW, N. Try to compose photos with mostly landscape and a minimal proportion of sky. Verify all photos and record the range of photo numbers in the logbook. Make sure photos

are in focus and have minimal glare (exclude the sun). Parts of the helicopter will end up in some of the photos, but try to minimize obstruction of the view if possible (for example, by moving to the other pontoon). The panoramic photos may be taken at any point during the sampling period.

II. SURFACE WATER SAMPLES

A. Vacuum pumped (screened) samples

Place a new 105 µm nitex screen on the vacuum sampler intake, save the bag and label it with the Station ID. Open the chamber, remove the insert and 1 L bottle, and rinse the insert in ambient water. Replace the insert, place and uncap the 1 L bottle, and reseal the chamber. Holding the intake at approximately 6" below the water surface, pump some water into the bottle (¼ full or so), remove and cap, shake to rinse, then dump the rinse away from the sampling area. Repeat the rinse a total of **3 TIMES**. Replace the 1 L bottle inside the chamber and pump to fill the bottle to the neck or shoulder (leaving room to shake). Cap the bottle and set it aside.

At the FIRST STATION OF THE DAY, collect an air deposition blank for mercury. After donning gloves (see below), open the field blank bottle, label it, and prop it up outside the helicopter with the cap tucked inside the bag. Leave the blank open during the time it takes for you to collect the mercury sample, then cap and reseal the bag for the blank. Change "clean hands" gloves to recap the air deposition blank if your original gloves get wet during sampling.

Put on mercury sampling gloves with a partner, and collect the mercury sample using the clean hands/dirty hands method. Note by initials in the logbook who was "clean hands" and who was "dirty hands." Gloves for "clean" are bagged in sets – one inner and one outer pair. Gloves for "dirty" are the regular sampling gloves, plus a pair of long gloves.

DIRTY: Rinse the mercury bottle chamber insert with ambient water and place in chamber.
Open outer bag of mercury sample bottle. *Do not touch the inner bag or bottle at any time.*
CLEAN: Open inner bag and remove bottle; tuck inner bag back inside outer bag.
DIRTY: Label bottle with Station ID.
CLEAN: Place bottle in insert inside chamber, *then* uncap it (hold the cap).
DIRTY: Close chamber, pump water to overflowing, open chamber.
CLEAN: Cap bottle, remove from chamber, place in inner bag and seal it.
DIRTY: Seal outer bag and place in black plastic bag inside the mercury cooler. Replace chamber insert.

Remove the nitex screen from the sampler intake, fold it, and place it back in the original, labeled bag. Place the bag on ice. Dump out any water from the chamber and drain the tubing.

B. Screened water samples

From the 1 L bottle of screened water (from A), subsample for the following parameters, in the containers with the label tape color indicated. Be sure to shake the screened water bottle before each subsample to ensure that it is well mixed.

Rinse the following bottles 3X with a small amount of sample (~¼ full), then fill to the neck:

- **BLUE** (TN/TP, TC) 125 mL poly (n=2)
- **GREEN** (SO_4/Cl) 125 mL poly
- **YELLOW** (DOM) 60 mL poly

Place all 4 of these bottles on ice.

Filter the screened water for the following samples as indicated. Make sure filters are not attached when removing the syringe plunger, to avoid rupturing the filter.

♦ **PINK** (nutrients) 60 mL poly

Using the 60 mL syringe, remove the plunger, and attach a new, individually-wrapped nylon syringe filter OR a swinnex filter assembly and nylon filter from the plastic box. Fill the syringe barrel with screened water, replace the plunger, rinse the bottle 3X with filtered water, then fill the bottle to the neck and place on ice. [NOTE: If using the swinnex assembly, dissemble and rinse it first in ambient site water (do not lose rubber gasket), load a new filter, then purge the filter with ~10 mL of the screened water to rinse the outlet area with filtered water.]

♦ **ORANGE** (DOC) 40 mL VOA

Using the same 60 mL syringe, refill the syringe barrel if necessary, and attach a new polysulfone filter to the syringe. Filter directly into the **pre-preserved** glass bottle (DO NOT RINSE), fill it to the top and cap it with no headspace. Try to avoid air bubbles if possible, but overfilling too much will dilute the preservative, so a few small bubbles may be present. Note that water spilling over the top will be acidic (pH<2). Put the bottle in the styrofoam rack inside the plastic bag. Seal the bag and place on ice. [**NOTE**: Remember to collect 3 of these at duplicate stations, each of the 2 times you collect samples, for a total of 6.]

C. Chlorophyll sample

Rinse the syringe, then draw water from 6" below surface into the 140 mL syringe. Avoid disturbing floating mats or epiphytic periphyton. Plunge out air and water to the 140 mL line. Attach a filter assembly pre-loaded with a GF/F filter to the syringe, and filter as much water through as possible without using excess force. Record the total volume filtered in the logbook. Remove the filter assembly, then draw ~50 mL air into the syringe. Reattach the assembly and plunge the syringe to remove all excess water. Unscrew the assembly, fold the filter twice using forceps, place in a microcentrifuge tube, and use the squeeze bottle to fill with 90% acetone. The filter should be completely submerged without air trapped in the bottom of the tube. Make sure you leave room to cap the tube without losing acetone. Label the tube with the Station ID and place it in the 500 mL dark brown plastic bottle on ice.

D. Bottom water sample

Choose a screen for the site, in the following order from fine to coarse pore size:

1 coffee filter 2 nitex screen 3 Henry sampler with sock 4 benthic bag cloth

Rinse the sampler and place a new screen/sock on the intake. Attach either short or long tubing (depending on water depth) to the top end of the sampler, and the syringe assembly with the 60 mL side syringe to the tubing. Attach a new pre-preserved **PURPLE** syringe to the assembly (save the cap), and make sure the valve is open towards the side syringe. Gently lower the bottom of the sampler to the sediment surface and use the SIDE syringe to remove air from the tube. Once you start drawing water into the side syringe, switch the valve to the **PURPLE** syringe and fill it to the 60 mL line. Close the valve, remove the side syringe and tubing, and check the syringe for air bubbles. Carefully purge any air bubbles out, then cap and place the syringe back in the long carrying case. Circle the screen/sampling method used on the field sheet. Remove the screen/sock/bag from the intake area and drain the tubing.

Place all used syringes, filters, screens, socks, cloth, etc. in the trash bag labeled with the Station ID, and place it in the same sample cooler with the samples. (Exception = the reusable 140 mL chlorophyll syringe and the nitex screen from the vacuum chamber that has already been labeled and placed on ice in Step A.)

III. SONDE DATA

Deploy the YSI sonde at 6" from the water surface and allow readings to stabilize. Log readings to the appropriate file on the sonde (not the display unit), then view the file to record readings in the logbook. To obtain the ORP (oxidation-reduction potential) measurement, first lower the sonde gently to the soil surface, resting the cage on the bottom so that it is standing upright. Allow to stabilize and record ORP measurement. Record the depth in tenths of a foot. This can be done simultaneously with water sample collection, provided you do not disturb the area where samples are being collected.

AFTER ENTERING WATER

At this point, crew members may enter the water, being careful not to disturb areas where soil cores and periphyton samples will be collected.

IV. DEPTH MEASUREMENTS

At 3 locations, measure water depth to the soil surface, then total depth to bedrock. Start with the **blue rod first**, then attach subsequent rods (which are numbered sequentially with the appropriate length) as needed. Allow rod to drop gently to the soil surface and record water depth, then slowly push into the soil until you hit bedrock and record total depth (from water surface to bedrock). The 3 measurement locations can all be within a meter or two of the pontoon, and should be at least a meter apart from each other. When removing rods from the soil, be sure to pull them straight up to avoid bending the screws holding sections together.

V. PERIPHYTON

A. Percent cover and composition

Find an area representative of the periphyton community at the station. Place the ¼ m^2 PVC quadrat randomly, by turning back towards the helicopter and tossing or dropping the frame behind you. This may require some adjustments in tall vegetation. Looking down into the quadrat, estimate the total % cover of periphyton using the estimation charts as a guide. This should be visualized as the percentage of quadrat area that would be covered by all types of periphyton if the three-dimensional quadrat volume was compressed flat into a two-dimensional plane. Take a photo of the quadrat, using the polarizer, from as close to nadir as possible while still including the quadrat frame. Verify the photo clarity and record the photo number in the logbook. Also record which of 5 categories of periphyton are present (circle Y or N for each one): floating mats, epiphytic "sweaters," benthic mats, green filamentous algae, and none/floc only (for where there is no visible periphyton).

B. Biovolume measurement (**A-Star crews ONLY**)

Rinse cylinders (and any other sampling/measuring vessels) with ambient site water before use. Harvest all periphyton within the three-dimensional quadrat region from water surface to soil, but exclude any benthic mat layer (to be collected from soil cores):

- **PF** (floating mat): skim off mat layer floating on water surface; strip from vegetation or wood
- **PE** (epiphytic "sweaters"): strip periphyton off stems of submerged plants or wood

Transfer all periphyton to an appropriately-sized graduated cylinder, depending on volume. The largest size is perforated to allow drainage. Use a spare tub to rinse and dislodge periphyton from fine-leaved plants if necessary. Strain dislodged material through a sieve and scrape off with a rubber spatula, then transfer it to a small graduated cylinder (or place on top of drained mats in the larger cylinder). Be careful not to lose these smaller pieces through the perforations. Measure the total volume (using multiple containers and/or refills if

necessary) and record it in the logbook. Transfer all measured periphyton to one tub and mix it by hand to homogenize.

C. Sample collection

A-Star Crews: Subsample the homogenized periphyton sample you collected in step B, by filling a labeled sample cup with a BLUE lid to the 120 mL line, then discard any remainder. If the total volume of the quadrat is <120 mL, add periphyton from the surrounding area (in representative proportions) to attain the 120 mL sample.

Jet Ranger Crews: Fill a labeled sample cup with a BLUE lid to the 120 mL line with periphyton from within the quadrat. Harvest PF and PE periphyton types by stripping off plants or wood, as described in step B above, but do not spend the time to wash very fine material off plants. Try to obtain 120 mL with minimal harvesting effort. If the volume obtained from the quadrat is <120 mL, add periphyton from the surrounding area (in representative proportions) to attain the 120 mL sample.

VIII. LOCATION

Set up the Trimble in the center of where the 3 soil cores will be collected. Follow the detailed instruction sheet to set up the file, and start logging points while collecting soil cores. Record the file name that is automatically generated by the unit. Record coordinates on the field sheet after completing the soil sampling. You need to log for a minimum of 20 minutes and obtain at least 36 points, but should ideally have several hundred.

VI. SOIL AND FLOC

A. Collect and measure cores

Rinse the corer and sleeve with site water. Assemble the corer with the appropriate handle and sleeve length for station conditions. Gently lower the core to the soil surface and mark the water level by sliding the ruler on the handle to align zero with the water surface. Slowly insert the corer to 10 cm (using a ruler or sliding marker as a guide), while turning the handle. DO NOT STOMP on the core top (to minimize compaction and ensure no loss of floc). Seal the corer by pulling the lever, then remove the core while holding one hand underneath once it is above the soil surface. Check that the soil core length = 10 cm (±0.5 cm); if not, repeat the process until you have 10 cm, unless the soil is shallow. You may need to account for submerged vegetation laying on top of the soil, by adding a few centimeters to the total depth needed. Record the core length (to the nearest tenth of a centimeter) in the logbook. Remove the handle attachment from the corer.

Take a photo of the intact core against the white background, with the wooden ruler next to the core itself. Verify the photo to ensure there is no backlighting of the core, and that it is in focus. Record the photo number in the logbook. Note the soil type(s) present in the logbook. Take photos of subsequent cores only if different than the first (for example, if the soil composition changes or if there is a change in the presence/absence of benthic periphyton).

B. Measure and collect floc and/or benthic periphyton

Measure and record thickness of floc (to the nearest centimeter) and/or periphyton (to the nearest tenth of a centimeter) in the logbook. Using the rubber stopper plunger, carefully push the core up through the sleeve to remove overlying surface water out the top. When you get to the floc layer, pour it into a storemore (plastic) bottle. Combine all 3 floc samples in one storemore. If you used a 2nd container or tub, record the total number

of each container type on the data sheet. If you do not collect all of the floc, record the estimated percentage that you did collect on the data sheet.

If benthic periphyton mats are present, remove them from the top of each core and place them all in one labeled sample cup with a WHITE lid. If all benthic mats do not fit into one sample cup, just fill it to the 120 mL line and discard any remainder (it is not necessary to homogenize and subsample).

C. Collect soil

Extrude soil core and combine all 3 cores (regardless of soil type) in a pre-weighed plastic bucket, label and seal. Rinse the corer and sleeve with site water, using a brush to dislodge soil in the threads if necessary.

VII. MOSQUITO FISH

Using the dip net, collect 15 mosquito fish (or as many as possible with reasonable time and effort). Place all fish in a bag labeled with the Station ID and add site water so that fish are suspended. Evacuate air when sealing the bag. Check the fish to verify that you have the correct species, then place the bag on ice.

IX. AERIAL PHOTOGRAPH

When leaving the site, take an aerial photograph of the site from 100-200 feet above. Have the pilot rotate the helicopter if necessary to get a clear view. Verify the photo and record the number in the logbook after landing at the next site.

AT LEAST ONCE PER DAY (by 2:00 pm), call in Station IDs with sample times.